

What is claimed is:

1. A method for producing a cytotoxic T-lymphocyte population primed for  
5 virus-specific CTL activity comprising the steps of:
  - (a) preparing non-naturally occurring antigen-presenting cells (nnAPC) which present at least one virus-specific antigen;
  - (b) harvesting a population of white blood cells from a subject;
  - (c) incubating a population of CD8+ cells obtained from the white blood  
10 cells in step (b) with the nnAPC cells; and
  - (d) treating the CD8+ cells with one or more supportive cytokines.
2. The method of claim 1 wherein the nnAPC cells present a plurality of the virus-specific antigens, and have been prepared by incubating the cells with at least  
15 two different peptides each comprising one of the virus-specific antigens, respectively.
3. The method of claim 1 or 2 further comprising incubating CD8+ cells from step  
20 (d) with non-proliferating peripheral blood mononuclear cell-derived adherent cells wherein the adherent cells present one or more of the same virus-specific antigens of step (a).
4. The method of any one of the preceding claims further comprising introducing  
25 at least one virus-inhibiting nucleic acid into the CD8+ cells.
5. The method of claim 4 wherein the virus-inhibiting nucleic acid is selected from the group consisting of transdominant proteins, intracellular antibodies, antisense molecules, RNA decoys, interfering RNAs, aptamers and ribozymes.
- 30 6. The method of claim 5 wherein the virus-inhibiting nucleic acid is a ribozyme.
7. The method of any one of claims 4 to 6 wherein the virus-inhibiting nucleic acid is specific for a disease selected from the group consisting of Human  
35 papilloma virus, Cytomegalovirus, Epstein Barr Virus, Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, Hepatitis E, Measles, Mumps, Polio, Rubella,

Influenza, Yellow Fever, Japanese Encephalitis, Dengue, Rabies, Rotavirus, Varicella Zoster, Chikungunya Rift Valley Fever, Respiratory Syncytial Virus, Herpes Simplex, Coronaviruses, Marburg, Ebola, California Encephalitis Virus, JC Virus, Lymphocytic Choriomeningitis Virus, Parvovirus, Rhinovirus, 5 Smallpox, HTLV-1, HTLV-2, and HIV.

8. The method of claim 7 wherein the virus-inhibiting nucleic acid is specific for HIV.

10 9. The method of any one of claims 4 to 8 wherein the virus-inhibiting nucleic acid is passed to CTL progeny.

10. The method of any one of claims 3 to 9 wherein the adherent cells are adherent monocytes obtained during the harvesting step (b).

15 11. The method of claim 10 wherein the adherent monocytes are isolated from a suspension of peripheral blood monocytes after irradiating the suspension with a sufficient dose of  $\gamma$ -radiation necessary to prevent proliferation of the peripheral blood monocytes.

20 12. The method of any one of claim 3 wherein the incubating step (c) comprises combining the CD8 $^{+}$  cells with the adherent peripheral blood monocytes at a ratio of about ten CD8 $^{+}$  cells to one adherent peripheral blood monocyte.

25 13. The method of any one of the preceding claims wherein the CD8 $^{+}$  cells are tested for at least one parameter selected from the group consisting of cytotoxic T cell activity, CTL cell purity, sterility and endotoxin content.

30 14. The method of any one of claims 1 to 13 wherein the supportive cytokines are selected from the group consisting of IL-2, IL-4, IL-7, IL-15 and IL-21.

35 15. The method of any one of the preceding claims wherein the supportive cytokines are added to the CD8 $^{+}$  cells in step (d) about 4 days or more after step (c) is initiated.

16. A method according to any one of claims 1 to 15 wherein the nnAPC cells comprise an nnAPC cell line.
- 5 17. A method for producing a cytotoxic T-lymphocyte population transduced with virus-inhibiting nucleic acid and primed for virus-specific CTL activity comprising the steps of:
  - (a) preparing a non-naturally occurring antigen presenting cell line (nnAPC) which presents at least one virus specific antigen;
  - (b) harvesting CD8<sup>+</sup> cells from a subject;
  - (c) incubating the CD8<sup>+</sup> cells with the nnAPC cell line;
  - (d) adding Interleukin-2 (IL-2) and Interleukin-7 (IL-7) to the CD8<sup>+</sup> cells after step (c);
  - (e) introducing at least one virus-inhibiting nucleic acid into the CD8<sup>+</sup> cells wherein the virus inhibiting nucleic acid is expressed; and
  - (f) incubating the CD8<sup>+</sup> cells with non-proliferating peripheral blood mononuclear cell-derived adherent cells and wherein the adherent cells present at least one of the same virus-specific antigens of step (a).
- 10 18. The method of claim 17 wherein the nnAPC cell line presents a plurality of virus-specific antigens, and have been prepared by incubating the cell line with at least two different peptides at least 8 amino acids in length, each peptide comprising one of the virus-specific antigens, respectively.
- 15 19. The method of claim 17 or 18 wherein the virus-inhibiting nucleic acid is a ribozyme.
20. The method of any one of claims 17 to 19 wherein the virus-inhibiting nucleic acid is specific for HIV.
- 30 21. The method of any one of claims 17 to 20 wherein the virus-inhibiting nucleic acid is passed to CTL progeny.
22. The method of claim 17 wherein the adherent cells are adherent monocytes obtained during the harvesting step (b).

23. The method of claim 22 wherein the adherent cells presenting at least one of the same virus-specific antigens of step (a) are produced by incubating the adherent cells with one or more different peptides, the or each peptide comprising one of the virus-specific antigens, respectively.

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24. The method of claim 22 or 23 wherein the adherent monocytes are isolated from a suspension of peripheral blood monocytes after irradiating the suspension with a sufficient dose of  $\gamma$ -radiation necessary to prevent further cell proliferation of the peripheral blood monocytes.

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25. The method of claim 24 wherein the dose of  $\gamma$ -radiation is in the range of about 3,000 to 7,000 rads.

15 26. The method of any one of claims 17 to 25 wherein the incubating step (f) further comprises combining the CD8 $^{+}$  cells with the adherent cells at a ratio of about ten CD8 $^{+}$  cells to one adherent cell.

27. The method of any one of claims 17 to 26 wherein the CD8 $^{+}$  cells are tested for at least one parameter selected from the group consisting of cytotoxic T cell activity, CTL cell purity, sterility and endotoxin content.

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28. The method of any one of claims 17 to 27 further comprising the step of introducing the CD8 $^{+}$  cells into a subject.

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29. The method of claim 28 wherein CD4 $^{+}$  T lymphocytes comprising virus inhibiting nucleic acid are also introduced into the subject.

30. The method of claim 28 wherein CD34 $^{+}$  hematopoietic progenitor cells comprising virus inhibiting nucleic acid are also introduced into the subject.

31. The method of claim 28 wherein both CD34 $^{+}$  hematopoietic progenitor cells comprising virus inhibiting nucleic acid and CD4 $^{+}$  T lymphocytes comprising virus inhibiting nucleic acid are also introduced into the subject.

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32. The method of any one of claims 28 to 31 wherein IL-2 is administered to the subject following the cell introduction step.
33. The methods of any of one of claims 17 to 32 wherein the subject tests positive for the presence of HIV antigen.
34. The method of claim 33 wherein antiretroviral therapy is stopped for a period of time following the introduction of the CD8+ cells into the subject.
35. A therapeutic cell product comprising a cytotoxic T-lymphocyte population primed for virus-specific CTL activity produced according to the method of any one of claims 1 to 16.
36. A therapeutic cell product comprising a cytotoxic T-lymphocyte population transduced with virus-inhibiting nucleic acid and primed for virus-specific CTL activity produced according to the method of any one of claims 17 to 27.
37. A method of treating a subject with an infectious disease, the method comprising administering to the subject a therapeutically effective dose of the therapeutic cell product of claim 35 or 36.
38. A method of treating a subject with an infectious disease, the method comprising:
  - (a) preparing non-naturally occurring antigen-presenting cells (nnAPC) which present at least one virus-specific antigen;
  - (b) harvesting a population of white blood cells from the subject;
  - (c) incubating a population of CD8+ cells obtained from the white blood cells in step (b) with the nnAPC cells;
  - (d) treating the CD8+ cells with one or more supportive cytokines; and
  - (e) introducing the CD8+ cells from step (d) into the subject.
39. The method of claim 38 wherein nnAPC cells present a plurality of the virus-specific antigens, and have been prepared by incubating the cells with at least two different peptides each comprising one of the virus-specific antigens, respectively.

40. The method of claim 38 or 39 further comprising incubating the CD8+ cells with non-proliferating peripheral blood mononuclear cell-derived adherent cells wherein the adherent cells present at least one of the same virus-specific antigenic peptides of step (a).

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41. The method of any one of claims 38 to 40 further comprising introducing at least one virus-inhibiting nucleic acid into the CD8+ cells, wherein the virus inhibiting nucleic acid is expressed in the lymphocytes.

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42. The method of claim 41 wherein the virus-inhibiting nucleic acid is selected from the group consisting of transdominant proteins, intracellular antibodies, antisense molecules, RNA decoys, interfering RNAs, aptamers and ribozymes.

15 43. The method of claim 42 wherein the virus-inhibiting nucleic acid is a ribozyme.

44. The method of any one of claims 38 to 43 wherein the virus-inhibiting nucleic acid is specific for a disease selected from the group consisting of Human papilloma virus, Cytomegalovirus, Epstein Barr Virus, Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, Hepatitis E, Measles, Mumps, Polio, Rubella, Influenza, Yellow Fever, Japanese Encephalitis, Dengue, Rabies, Rotavirus, Varicella Zoster, Chikungunya Rift Valley Fever, Respiratory Syncytial Virus, Herpes Simplex, Coronaviruses, Marburg, Ebola, California Encephalitis Virus, JC Virus, Lymphocytic Choriomeningitis Virus, Parvovirus, Rhinovirus, Smallpox, HTLV-1, HTLV-2, and HIV.

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45. The method of claim 44 wherein the virus-inhibiting nucleic acid is specific for HIV.

30 46. The method of any one of claims 41 to 45 wherein the virus-inhibiting nucleic acid is passed to CTL progeny.

47. The method of claim 38 wherein the adherent cells are adherent monocytes obtained during the harvesting step (b).

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48. The method of claim 47 wherein the adherent cells presenting at least one of the same virus-specific antigenic peptides of step (a) are produced by incubating the adherent cells with one or more different peptides, the or each peptide comprising one of the virus-specific antigens, respectively.

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49. The method of claim 47 or 48 wherein the adherent monocytes are isolated from a suspension of peripheral blood monocytes after irradiating the suspension with a sufficient dose of  $\gamma$ -radiation necessary to prevent further cell proliferation of the peripheral blood monocytes.

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50. The method of claim 49 wherein the dose of  $\gamma$ -radiation is in the range of about 3,000 to 7,000 rads.

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51. The method of claim 40 wherein the incubating step (c) further comprises combining the CD8 $^{+}$  cells with the adherent peripheral blood monocytes at a ratio of about ten CD8 $^{+}$  cells to one adherent peripheral blood monocyte.

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52. The method of any one of claims 38 to 51 wherein the CD8 $^{+}$  cells are tested for at least one parameter selected from the group consisting of cytotoxic T cell activity, CTL cell purity, sterility and endotoxin content.

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53. The method of any one of claims 38 to 40 further comprising incubating a population of CD4 $^{+}$  T lymphocytes obtained from the white blood cells in step (b) with the nnAPC cells separately from the CD8 $^{+}$  cells, and introducing the CD4 $^{+}$  T lymphocytes into the subject.

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54. The method of claim 53 further comprising adding one or more supportive cytokines to the CD4 $^{+}$  T lymphocytes prior to introducing the T lymphocytes into the subject..

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55. The method of claim 53 or 54 further comprising introducing a virus-inhibiting nucleic acid into the population of CD4 $^{+}$  T lymphocytes prior to introducing the T lymphocytes into the subject, and wherein the virus inhibiting nucleic acid is expressed in the lymphocytes.

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56. The method of any one of claims 38 to 40 further comprising incubating a population of CD34+ haematopoietic progenitor cells with the nnAPC cells separately from the CD8<sup>+</sup> cells for a period of time to stimulate the CD34<sup>+</sup> cells prior to introducing the CD34<sup>+</sup> cells into the subject.

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57. The method of claim 56 further comprising adding one or more supportive cytokines to the CD34+ haematopoietic progenitor cells prior to introducing the CD34<sup>+</sup> cells into the subject.

10 58. The method of claim 56 or 57 further comprising introducing a virus-inhibiting nucleic acid into the population of CD34+ haematopoietic progenitor cells prior to introducing the CD34<sup>+</sup> cells into the subject, and wherein the virus inhibiting nucleic acid is expressed in the CD34<sup>+</sup> cells.

15 59. The method according to any one of claims 38 to 58, wherein both CD34<sup>+</sup> hematopoietic progenitor cells comprising virus inhibiting nucleic acid and CD4<sup>+</sup> T lymphocytes comprising virus inhibiting nucleic acid are also introduced into the subject.

20 60. The method of any one of claims 38 to 52 wherein the supportive cytokines are selected from the group consisting of IL-2, IL-4, IL-7, IL-15 and IL-21.

61. The method of claim 60 wherein the supportive cytokines are IL-2 and IL-7.

25 62. The method of any one of claims 38 to 61 wherein the CD8<sup>+</sup> cells are incubated with the nnAPC cells for a period of from about 5 to 7 days.

63. The method of any one of claims 38 to 62 wherein the supportive cytokines are added to the CD8+ cells about 4 days or more after step (c) is initiated.

64. The method of any one of claims 38 to 63 wherein the subject has more than one infectious disease, and the nnAPC cells present at least one virus-specific antigen for each disease, respectively.

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65. A method according to any one of claims 38 to 64 wherein the nnAPC cells comprise a cell line.